

The invention claimed is:

1. A method for identifying the 3'-end of a polynucleotide appearing as a chromatographic peak in a first IP-RP-HPLC chromatogram, wherein said polynucleotide has been generated by subjecting a precursor nucleic acid of known sequence to a first cleavage reaction, and wherein said polynucleotide shares with said precursor nucleic acid a common 5'-end, the method comprising:

(a) providing a plurality of polynucleotides, wherein said plurality of polynucleotides have been generated by subjecting the precursor nucleic acid of known sequence to a second cleavage reaction, wherein said second cleavage reaction is base-discriminating and is distinct from the first cleavage reaction, and wherein said plurality of polynucleotides share with said precursor nucleic acid and with each other a common 5'-end ;

(b) separating said plurality of polynucleotides by IP-RP-HPLC, wherein the IP-RP-HPLC separation conditions are substantially the same as those used to generate said first IP-RP-HPLC chromatogram;

(c) detecting said plurality of polynucleotides as they elute from the IP-RP-HPLC separation, thereby generating a second HPLC chromatogram, wherein the 3'-end of a polynucleotide appearing as a chromatographic peak in said second IP-RP-HPLC chromatogram can be determined based on the known sequence of the precursor nucleic acid; and

(d) comparing said first IP-RP-HPLC chromatogram with said second IP-RP-HPLC chromatogram, wherein the 3'-end of a polynucleotide appearing as a chromatographic peak in said first IP-RP-HPLC chromatogram can be identified based on its elution position relative to a peak appearing in said second IP-RP-HPLC chromatogram whose 3'-end is known.

2. The method of Claim 1, wherein said IP-RP-HPLC employs a separation medium that is substantially free of multivalent cations that are capable of interfering with polynucleotide separations.

3. The method of Claim 2, wherein said polynucleotide is DNA.

4. The method of Claim 3, wherein said separation medium comprises particles selected from the group consisting of silica, silica carbide, silica nitrite, titanium oxide, aluminum oxide, zirconium oxide, carbon, insoluble polysaccharide, and diatomaceous earth, the particles having separation surfaces which are coated with a hydrocarbon or non-polar hydrocarbon substituted polymer, or have substantially all polar groups reacted with a non-polar hydrocarbon or substituted hydrocarbon group, wherein said surfaces are non-polar.

5. The method of Claim 3, wherein said separation medium comprises polymer beads having an average diameter of 0.5 to 100 microns, said beads being unsubstituted polymer beads or polymer beads substituted with a moiety selected from the group consisting of hydrocarbon having from one to 1,000,000 carbons.

6. The method of Claim 5, wherein said beads are substituted with a moiety selected from the group consisting of methyl, ethyl, or hydrocarbon having from 23 to 1,000,000 carbons.

7. The method of Claim 3, wherein said separation medium comprises a monolith.

8. The method of Claim 3, wherein said separation medium has been subjected to acid wash treatment to remove any residual surface metal contaminants.

9. The method of Claim 3, wherein said separation medium has been subjected to treatment with a multivalent cation binding agent.

10. The method of Claim 3, wherein said IP-RP-HPLC employs a mobile phase

comprising a solvent selected from the group consisting of alcohol, nitrile, dimethylformamide, tetrahydrofuran, ester, ether, and mixtures of one or more thereof.

11. The method of Claim 10, wherein said mobile phase comprises acetonitrile.

12. The method of Claim 3, wherein said mobile phase comprises a counterion agent

5 selected from the group consisting of lower alkyl primary amine, lower alkyl secondary amine, lower alkyl tertiary amine, lower trialkylammonium salt, quaternary ammonium salt, and mixtures of one or more thereof.

13. The method of Claim 12, wherein said counterion agent is selected from the group

10 consisting of octylammonium acetate, octadimethylammonium acetate, decylammonium acetate, octadecylammonium acetate, pyridiniumammonium acetate, cyclohexylammonium acetate, diethylammonium acetate, propylethylammonium acetate, propyldiethylammonium acetate, butylethylammonium acetate, methylhexylammonium acetate, tetramethylammonium acetate, tetraethylammonium acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, dimethyldiethylammonium
15 acetate, triethylammonium acetate, tripropylammonium acetate, tributylammonium acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, triethylammonium hexafluoroisopropyl alcohol, and mixtures of one or more thereof.

14. The method of Claim 13, wherein said counterion agent is tetrabutylammonium acetate.

20 15. The method of Claim 13, wherein said counterion agent is triethylammonium acetate.

16. The method of Claim 12, wherein said counterion agent includes an anion, said anion is selected from the group comprising acetate, carbonate, phosphate, sulfate, nitrate, propionate, formate, chloride, and bromide.

17. The method of Claim 3, wherein said detection is achieved using Matched Ion Polynucleotide Chromatography.

18. The method of Claim 3, wherein said polynucleotides are detectably labeled.

19. The method of Claim 18, wherein said detectable label is fluorescent.

5 20. The method of Claim 19, wherein said detectable label is selected from the group consisting of FAM, JOE, TAMRA, ROX, HEX, TET, Cy3, and Cy5.

21. The method of Claim 20, wherein said detectable label is FAM.

22. The method of Claim 3, wherein the method is used to identify the 3'-ends of a plurality of polynucleotides appearing as chromatographic peaks in said first IP-RP-HPLC
10 chromatogram by comparing said first IP-RP-HPLC chromatogram and said second IP-RP-HPLC chromatogram.

23. The method of Claim 3, wherein said first cleavage reaction involves the use of a DNA cleavage reagent that cleaves DNA that is not protected by a bound protein.

24. The method of Claim 3, wherein said cleavage reagent is a hydroxyl radical.

15 25. The method of Claim 3, wherein said cleavage reagent is a nuclease.

26. The method of Claim 25, wherein said nuclease is DNase I.

27. The method of Claim 23, wherein said protein is a mismatch binding protein.

28. The method of Claim 27, wherein said mismatch binding protein is selected from the group consisting of T4 endonuclease VII, T7 endonuclease I, S1 nuclease, mung bean
20 endonuclease, MutY protein, MutS protein, MutH protein, MutL protein, cleavase, and CELI.

29. The method of Claim 28, wherein said mismatch binding protein is CELI.

30. The method of Claim 23, wherein said protein is a protein involved in DNA transcription, replication, and recombination.

31. The method of Claim 23, wherein said protein is selected from the group consisting of transcription factors, enhancers and repressors.

32. The method of Claim 23, wherein said protein binds to a Holliday junction.

33. The method of Claim 24, wherein said protein is RuvA.

5 34. The method of Claim 3, wherein said second cleavage reaction is a reaction that results in the specific cleavage of a DNA sequence preferentially after one, two or three of the bases selected from the group consisting of adenine, guanine, cytosine, and thymine.

35. The method of Claim 34, wherein said second cleavage reaction is a chemical cleavage DNA sequencing reaction.

10 36. The method of Claim 35, wherein said sequencing reaction is an A+G specific DNA sequencing reaction.

37. The method of Claim 36, wherein said DNA sequencing reaction is based on partial acidic hydrolyses of DNA in the presence of diphenylamine and proceeds via depurination/5',3'-elimination.